HHS Public Access

Author manuscript

Anal Bioanal Chem. Author manuscript; available in PMC 2016 April 19.

Published in final edited form as:

Anal Bioanal Chem. 2014 August ; 406(21): 5187–5194. doi:10.1007/s00216-014-7718-7.

An Enhanced Butyrylcholinesterase Method to Measure Organophosphorus Nerve Agent Exposure in Humans

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Abstract

Organophosphorus nerve agent (OPNA) adducts to butyrylcholinesterase (BChE) can be used to confirm exposure in humans. A highly accurate method to detect G-series and V-series OPNA adducts to BChE in 75 μL of filtered blood, serum, or plasma has been developed using immunomagnetic separation (IMS) coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS). The reported IMS method captures > 88% of the BChE in a specimen and corrects for matrix effects on peptide calibrators. The optimized method has been used to quantify baseline BChE levels (unadducted and OPNA-adducted) in a matched set of serum, plasma and whole blood (later processed in-house for plasma content) from 192 unexposed individuals to determine the interchangeability of the tested matrices. The results of these measurements demonstrate the ability to accurately measure BChE regardless of the format of the blood specimen received. Criteria for accepting or denying specimens were established through a series of sample stability and processing experiments. The results of these efforts are an optimized and rugged method that is transferrable to other laboratories and an increased understanding of the BChE biomarker in matrix.

Keywords

Organophosphorus nerve agent; butyrylcholinesterase; cholinesterase inhibitors; protein adduct; immunomagnetic separation

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Author Contributions

The manuscript was written with contributions from all authors. All authors have given approval to the final version of the manuscript.

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Introduction

The threat of toxic organophosphorus nerve agent (OPNA) use has led to the development of bioanalytical methods to detect G-series and V-series OPNA exposure in humans. The Chemical Weapons Convention, administered by the Organisation for the Prohibition of Chemical Weapons, classifies OPNA compounds as Schedule 1 chemical warfare agents since they are solely used as chemical weapons [1]. Organophosphorus (OP) compounds, including nerve agents and pesticides, inhibit cholinesterases (ChE) by phosphorylating serine in the enzymatic active site resulting in over-stimulation of cholinergic receptors [2]. ChE inhibition causes symptoms ranging from muscle twitching and seizures to death in high level exposures [3, 4]. OP agents, specifically sarin (GB), were used as weapons in the Matsumoto City and Tokyo subway attacks [4], the Iraq-Iran conflict [5] and most recently in Syria [6]. Hence, reliable methods for immediate and retrospective detection of OPNA exposure are essential.

Historically, the Ellman assay has been the primary method to diagnose OP exposure in clinical samples. The Ellman assay measures erythrocyte acetylcholinesterase (AChE) activity using a kinetic colorimetric system [7] and has been adapted to measure butyrylcholinesterase (BChE) [8]. Despite its advantages, the Ellman assay is unable to identify specific OP compounds, is unreliable at inhibition levels <20%, and is unsuitable for retrospective detection due to normal restoration of enzyme activity [9, 10].

Other approaches for detecting OPNA exposure include the measurement of OPNA hydrolysis products in urine [12], OPNA fluoride reactivation in blood [11] and OPNA adduction to albumin in blood [18]. OPNA metabolites including GB, soman, cyclosarin, VX and Russian-VX phosphonic acids are rapidly detected in urine using liquid chromatography tandem mass spectrometry (LC-MS/MS) [12, 13]. A significant limitation of the method is that > 90% of these hydrolysis products are eliminated *in vivo* within 2–3 days after exposure [14]. Fluoride reactivation can detect low level nerve agent exposure up to 35 days post OP exposure [15]. The limitations of fluoride reactivation include its inability to measure endogenous BChE levels or aged OPNA adducts, and spontaneous reactivation and denaturation of the enzyme which affect quantitative measurements [16]. Albumin is a common protein biomarker since nerve agents covalently bind to tyrosine-411 of albumin. Unlike other protein biomarkers, tyrosine-411 of albumin does not ‘age’ or degrade in the presence of oximes [17]. Furthermore, albumin is the most abundant protein in blood plasma (40 mg/mL) and has a relatively long half-life of 20 days [18,19]. However, albumin reacts 500 times slower with nerve agents in blood than cholinesterases [19].

BChE is a prominent OPNA target [18, 20]. BChE acts as a natural scavenger of OPNA [21], is easily extracted from blood plasma, has a high rate of reaction [22], and is vastly more abundant in serum than AChE [20]. BChE adducts are detectable up to 16 days post-exposure making them an effective biomarker for retrospective detection [23].

The BChE diagnostic method and immunomagnetic separation (IMS) coupled with isotope dilution LC-MS/MS method was originally developed by The Netherlands Organisation for Applied Scientific Research (TNO) and Knaack *et al.* [16, 24]. In brief, BChE is extracted

from the specimen using anti-BChE antibody (Ab) conjugated to ferromagnetic Protein G beads. Upon extraction, BChE is digested using pepsin and analyzed by LC-MS/MS (Fig. 1). MS analysis readily reveals whether a nine amino acid peptide formed by pepsin digestion, $FGES_{198}AGAAS$ (where S_{198} is the active site serine), contains the OP adduct to the serine-198 residue [16, 24].

The reported method has been modified significantly to enhance accuracy and to expand our knowledge of BChE in matrix. To optimize the IMS-LC-MS/MS method, GB and VX peptides were used as model analytes to represent G-series and V-series OPNAs, respectively. V-series agents signify long persistence, unlike G-series which are non-persistent. The new method quantifies unadducted BChE, GB-adducted BChE (GB-BChE) and VX-adducted BChE (VX-BChE) in human serum and plasma samples. Endogenous BChE protein levels were measured in a matched-set of 192 individual unexposed serum, plasma, and plasma harvested from whole blood samples. BChE sample handling and stability experiments were conducted. The result of these efforts is a highly accurate and rugged method for detection of OPNA exposure in humans.

Materials and Methods

Reagents and Materials

Material sources were as follows: anti-butyrylcholinesterase monoclonal antibody from clone 3E8, KingFisher deep 96-well plates, KingFisher shallow 96-well plates and Easy Pierce Heat Sealing foil (Fisher Scientific); pepsin from porcine gastric mucosa, PBS with Tween-20, formic acid (~98% purity) (Sigma-Aldrich Chemical Company); HPLC-grade water and acetonitrile (Tedia); MultiScreen 96-well 0.45 μm PVDF plates (EMD Millipore); 96-well PCR plates, adhesive PCR foil (Eppendorf); protein precipitation plates (Pierce, Rockford, IL); Dynabeads Protein G (Invitrogen); Aquasil C18 HPLC column (1.0 × 50 mm, 3 μm) (Thermo Scientific). Native and labeled synthetic peptides (>58% peptide content) were synthesized at TNO. Pooled serum for quality controls (QC) was purchased from Bioreclamation and spiked with solvent, GB or VX agent at Battelle Memorial Institute.

Convenience Sample Set

A convenience set containing matched-set serum, plasma and whole blood specimens from 192 healthy individuals with no known exposure to nerve agents was purchased from Tennessee Blood Services. Only donor age, sex, and smoking status were provided; therefore, no institutional review board approval was required. Baseline BChE protein levels and protein stability was assessed among the three matrices using the reported IMS-LC-MS/MS method. BChE protein stability experiments were conducted over 2 weeks using pooled convenience set samples stored between 37°C to −70°C.

Synthetic Calibrators and Quality Controls

The peptide calibration curve was comprised of a mixture of native unadducted BChE [FGESAGAAS], GB-BChE [FGES(-GB)AGAAS] and VX-BChE [FGES(-VX)AGAAS] synthetic peptides. The calibration curve was comprised of 8 calibrators at 1, 4, 6, 16, 32,

63, 125, and 250 ng/mL in 0.6% formic acid in HPLC-grade water. The calibration range corresponded with the range of BChE concentrations observed from the convenience set (13.8–58.3 ng/mL). The lowest point on the calibration curve is the lowest reportable limit (LRL) for GB-BChE and VX-BChE, while BChE had a LRL of 4 ng/mL. The highest point on the calibration curve is above the range of expected results. The internal calibrator was comprised of a mixture containing $^{13}C_9$-isotopically labeled BChE, GB-BChE and VX-BChE synthetic peptides in 0.6% formic acid in HPLC-grade water. No isotopic contributions between the labeled and native peptides were observed. Peptide content was measured in triplicate by MidWest Biotech using amino acid analysis to provide accurate calibrator and internal standard concentrations.

Quality control materials included three QC high (QCH), one QC low (QCL) and one matrix blank serum sample. The QCH samples were 100% unadducted BChE (QCH BChE); 100% GB-adducted BChE (QCH GB); and 100% VX-adducted BChE (QCH VX). The QCL material was prepared as a single mixture comprised of 5 parts QCH BChE, 2 parts QCH GB and 1 part QCH VX. A small excess of unadducted VX in the QCH VX serum requires additional QCH BChE serum to scavenge the free VX when preparing the QCL mixture. The matrix blank QC material was prepared from 100% GA-adducted BChE (Battelle) since GA is not a current analyte in the method due to the unavailability of appropriate synthetic peptide calibrators.

Preparation of Specimens

At least one day prior to sample processing, anti-BChE Ab was conjugated to the magnetic Protein G Dynabeads as described by Knaack *et al.* [24]. Once a stock of anti-BChE beads were prepared, a 125 µL aliquot of the five QCs and specimens were filtered through a 96-well 0.45 µm filter plate at 3,000 ×g at 20°C to remove particulates. Whole blood samples (including the convenience set) were centrifuged at 3,000 ×g at 20°C to harvest plasma. Unfiltered blood was not processed through the method since the quantitative values for the unfiltered blood were greater than ± 20% of the theoretical. A 75 µL aliquot of the filtered specimen was transferred to a deep 96-well plate (sample plate). To address matrix effects, 75 µL of filtered matrix blank QC material was added to wells that would later receive the calibration curve. In a separate shallow 96-well plate, 50 µL of anti-BChE beads were added to the same number of wells as samples to be measured.

Sample Processing

Using a KingFisher Flex Magnetic Particle Processor (Thermo Scientific), the anti-BChE beads were transferred to the specimens in the sample plate. The sample plate was covered with adhesive foil, placed on the Eppendorf MixMate and shaken at 1,400 rpm for 2 hours at room temperature. During the sample plate incubation, the following were prepared: pepsin solution, three wash plates and the digestion plate. The 2 mg/mL pepsin solution in 0.6% formic acid in HPLC-grade water was made at least 30 min prior to use. Wash plates were made by adding 500 µL of PBS with Tween-20 to each well of three deep 96-well plates. The digestion plate was prepared by adding 10 µL internal calibrator to each well of a shallow 96-well plate. Each calibrator (75 µL of 1, 4, 6, 16, 32, 63, 125 and 250 ng/mL native BChE, GB-BChE and VX-BChE peptides) was added to the appropriate wells on the

digestion plate. The remaining wells without the calibration curve had 75 μL 0.6% formic acid in HPLC-grade water added to them. Immediately prior to the extraction of the BChE protein, 10 μL of 2 mg/mL pepsin solution was added to every well of the digestion plate.

The sample plate, digestion plate, and three wash plates were loaded onto the KingFisher Flex to capture the bead bound BChE. The digestion plate was covered with adhesive foil, placed on the Eppendorf Thermomixer R for 30 minutes at 37°C with intermittent shaking at 1,000 rpm for 10 seconds every minute.

Using the KingFisher Flex, the anti-BChE beads were removed from the digestion plate, leaving behind the pepsin digested sample. A 285 μL aliquot of acetonitrile and the digested samples were added to a protein precipitation plate. The protein precipitation plate was shaken for 1 minute at 1,000 rpm. The digested samples were filtered through the protein precipitation plate using a vacuum manifold, eluting the sample into the deep 96-well plate. The eluent was dried using a TurboVap-96 (Biotage) at 70°C under a nitrogen stream. The samples were reconstituted with 75 μL 0.6% formic acid in HPLC-grade water, transferred to a 96-well PCR plate, and heat sealed.

The BChE biomarker peptides were analyzed on an AB Sciex 6500 triple quadruple mass spectrometer interfaced with an Agilent 1290 Infinity series HPLC. Data was collected with Analyst 1.6 (AB Sciex) using Multiple Reaction Monitoring (MRM), with a dwell time of 15 ms for each of the nine transitions, as summarized in Figure 2a. The TurboIonSpray source operated in positive ion mode and under the following parameters: curtain gas= 10 psi; collision gas= 10 psi; ionspray voltage= 5500 V; temperature= 400°C; ion gas source 1= 20 psi and ion gas source 2= 10 psi. The collision energy and declustering, entrance, and collision cell exit potentials were optimized for each transition and are reported in Figure 2a. The fragmentation patterns of these peptides have been previously reported [16]. The peptide extract (10 μL) was separated by reversed-phase chromatography using an Aquasil C18 HPLC. Mobile phases were 0.1% formic acid in HPLC-grade water (mobile phase A) and aqueous acetonitrile (mobile phase B). A segmented gradient was run over 5 minutes and at a flow rate of 75 μL/min as previously described [16]. The segmented gradient yielded elution times of 1.86 – 2.11 (± 0.2min) for all analytes. Extracted ion chromatograms were assessed on Analyst 1.6 for correct analyte peak shape, retention time and manually integrated when needed (Fig. 2b–c).

Validation/Characterization

QC characterization/validation was comprised of 20 analytical runs performed by three analysts (an analytical run contains one calibration curve in matrix blank and five QC materials) and 192 human samples. A maximum of two runs were prepared and analyzed per day over the span of five months. Linearity, precision and accuracy were determined and the limits of detection were calculated using the Taylor method [25]. Matrix effects were addressed by comparing non-matrix and matrix based calibrators. Ruggedness was assessed by altering the method's parameters and measuring the deviations in accuracy.

Results and Discussion

Linearity and Sensitivity

Following the completion of the 20 characterization runs, statistical analysis was conducted to validate the IMS method. Calibration curves were linear for all three analytes with a coefficient of determination value >0.99. The linear range was 4–250 ng/mL for unadducted BChE and 1–250 ng/mL for GB-BChE and VX-BChE. The limits of detection (LOD) and limits of quantitation (LOQ) were calculated using the Taylor method, in which 3 calibrators (low, mid and high) were analyzed for standard deviation (SD). The SD was plotted against the respective concentration and calculated as 3 times (LOD) or 10 times (LOQ) the y-intercept [25]. The calculated LOD values for the analytes, BChE, GB-BChE and VX-BChE were 1.42, 0.79 and 0.43 ng/mL, respectively for the quantitation ions. The lowest calibrator for each analyte was higher than the calculated LOD. Precision was determined by calculating the mean percent relative standard deviation (%RSD) of the eight calibrators through the 20 analytical runs. The mean %RSD for the 8 calibrators were 8.1%, 6.1% and 5.4%, corresponding to BChE, GB-BChE and VX-BChE native peptides (Table 1).

Peptide Stability and Recovery

Peptide temperature and freeze-thaw stability were evaluated by spiking the peptides into processed matrix blank. Both native and $^{13}C_9$-isotopically labeled peptides were stable for at least 2 months at room temperature and greater than six months when stored at −70°C. Peptide calibrators suspended in 0.6% formic acid in HPLC-grade water were subjected to four freeze-thaw cycles from −70°C to 22°C and found to be stable. For both the temperature and the freeze-thaw stability cycles, the peptides were found to be within ± 10% of the theoretical.

Peptide recovery was measured by comparing the peak area of unprocessed calibrators to the peak area of processed calibrators. The unprocessed calibrators were added immediately before LC-MS/MS analysis (n=5) while the processed samples were added prior to pepsin digestion (n=5). Peptide recovery was 80± 14%, 74± 8%, and 75± 8% for BChE, GB-BChE and VX-BChE native peptides, respectively. To examine potential sources of peptide loss, peptide digestion and possible peptide adhesion to the wells was investigated. Ultimately the decrease in peptide recovery was not from the over- or under-digestion of the peptide. A slight decrease in peptide signal due to the adhesion of certain peptides to the wells was observed when compared to samples resuspended in low binding protein plates.

Quality Control Statistics

QC materials were evaluated according to amended Westgard rules [26, 27]. Analytical results were considered “out-of-control” if a QC control fell outside of the 3 SD confidence interval. The second parameter for QC exclusion occurred if two consecutive QC results fell outside of the 2 SD confidence interval. The mean calculated concentrations and %RSD for unadducted BChE, GB-BChE and VX-BChE QC materials are shown in Table 2.

BChE Protein Recovery

Optimization efforts resulted in an increase in BChE protein recovery. Knaack *et al.* previously reported capturing 53.4% of the BChE protein using the anti-BChE beads [24]. By increasing the ratio of beads to serum by 3-fold, the optimized IMS-LC-MS/MS method captures ≥40% more BChE protein compared to the previous method (Table 3). BChE protein extraction efficiency was determined by analyzing $FGES_{198}AGAAS$ peptide levels from QCH BChE, QCH GB and QCH VX serum QC materials that were depleted three times with BChE Ab-conjugated beads. As a result, ≥88% of the BChE protein was extracted during the first incubation with the beads. Exhaustive BChE protein extraction occurred with three depletions.

IMS-LC-MS/MS Method Optimization

To improve robustness, several key parameters were tested and optimized. Native and labeled peptides were added prior to pepsin digestion instead of spiked at the end of the method in order to account for losses during sample preparation. This was the earliest point of addition as anti-BChE beads would not capture the peptide calibrators. Matrix effects were assessed by comparing matrix and solvent-based (i.e. 0.6% formic acid in HPLC-grade water) calibration curves. The slopes between the two matrices were greater than 5% different; therefore, the slopes were not equivalent and a matrix-based curve is required for accurate quantitation. Sample preparation time was decreased by adjusting the digestion conditions and peptide clean-up process. The BChE protein was fully digested into the nine amino acid peptide, $FGES_{198}AGAAS$, within 30 minutes, reducing the digestion time from 2 hours. The digestion reaction was quenched by adding acetonitrile and filtered through a protein precipitation plate. As a result of the digestion and filtration modifications, the sample preparation time reduced from 8 to 4 hours.

The reported method requires only 75 μL of filtered sample. By increasing BChE protein recovery, the sample volume was reduced 4-fold without decreasing sensitivity. The lower sample volume requirement allows for potential pediatric samples and limited sample volumes that would be expected in an OPNA chemical emergency. Due to the limitations of the previous method, results were reported as percent adducted/unadducted BChE. The current method allows for the direct reporting of quantitative values of unadducted BChE, GB-BChE and VX-BChE nine amino acid peptide.

During ruggedness testing several conditions were monitored including, digestion time and temperature, enzyme concentration, and sample concentration with nitrogen drying. Reducing digestion time to less than 30 minutes resulted in the under-digestion of BChE. Other actions that did not affect BChE were increasing the digestion times up to 2 hours, increasing pepsin concentration 4-fold, and varying the drying temperatures under nitrogen pressure between 37°C to 70°C.

BChE Protein Stability and Handling

Baseline BChE levels in the 192 matched plasma, serum and blood specimens were analyzed to compare results among the matrices. Establishing baseline BChE levels is essential since BChE is endogenous in >99.9% of the population [20]. Values outside 1 SD

of the average BChE concentration for healthy unexposed individuals could be “flagged” for potential OP exposure. A cut-off of 1 SD increases the number of samples that will have to be tested but avoids false negatives. Based on this premise, a high throughput prioritization method to profile ChE adducts was recently developed [28].

During the shipment of the convenience set samples, red blood cell lysis was indicated in the whole blood specimens by the discoloration of the harvested plasma samples. Despite lysis of the red blood cells, plasma and blood produced similar BChE results and therefore, can be used interchangeably. The BChE concentrations in the serum, plasma, and plasma harvested in-house from whole blood were 34.8 ± 7.8 ng/mL, 34.0 ± 9.2 ng/mL and 36.0 ± 7.7 ng/mL (mean concentration ± SD), respectively. As a result, these three matrices can be analyzed interchangeably for BChE quantification (Table 4).

Proper handling procedures and criteria for accepting or denying specimens were established by quantifying BChE levels under a variety of storage and sample handling conditions. Proper sample handling procedures have been previously established by the Clinical and Laboratory Standards Institute (CLSI) [29]. However, specimens received during an emergency may not always be acquired and/or stored under proper conditions prior to delivery. Blood, serum and plasma were collected from pooled convenience set samples. Pooled samples were aliquoted and immediately analyzed (t= 0 days) or stored at 37°C, 22°C, 4°C, −20°C or −70°C. Triplicate plasma, serum and blood aliquots at each temperature were analyzed immediately or after 1, 7 or 14 days. The BChE protein in plasma, serum, and blood was found to be stable up to 2 weeks when stored between 37°C to − 70°C. When frozen, whole blood was severely lysed and required centrifugation through a 0.45 um filter prior to analysis.

Aged OP-adducted BChE

Despite these improvements, the reported method is unable to measure aged adducts. To address aged OP adducts, a simultaneous method to quantify the methylphosphonic acid (MeP) adduct to serine-198 on BChE was developed [30]. The MeP adduct is the product of aged V- and G- series OPNAs having a direct phosphorus-methyl bond. Although MeP is specific to OPCW Schedule 1 chemicals in general and not the exact OPNA of exposure, it is chemically stable and allows for the retrospective detection of aged OPNA adducts which contain the signature phosphorus-methyl bond. An alternate method to quantify OPNA adducts to tyrosine-411 on albumin following BChE depletion is currently under development in this laboratory.

Conclusion

In summary, an optimized method to confirm G- and V-series organophosphorus nerve agent exposure in human serum, plasma, and blood by quantifying butyrylcholinesterase biomarker peptides has been developed. The recent modifications have decreased sample volume requirements significantly without influencing LC-MS/MS method or limits of detection. In addition, the reported method reduces sample processing time by half and has endured ruggedness testing.

The stability of BChE and baseline BChE levels among various matrices has been demonstrated. Criteria for the acceptance of clinical samples for this method exceed the recommendations provided by CLSI and include considerations such as improper sample handling and storage. Plasma, serum, and plasma harvested from whole blood can be analyzed interchangeably using this method. Specimen stability experiments showed that plasma, serum, and blood samples stored at 37°C or colder for two weeks are stable, but whole blood samples need to be filtered if frozen.

Acknowledgments

Funding Information

This work was funded by the Centers for Disease Control and Prevention, Defense Threat Reduction Agency and Oak Ridge Institute for Science and Education.

The authors would like to thank the members of the Analytical Response and Chemical Laboratory Response Network Laboratories for technical assistance and thoughtful discussion during the preparation of this manuscript. Specifically, the authors would like to thank Ms. Chariety Sapp of the Centers for Disease Control and Prevention's Incident Response Laboratory for aliquoting and distributing the convenience set specimens and Ms. Karen Shields for thoughtful input to the manuscript.

Abbreviations

OPNA	organophosphorus nerve agents
BChE	butyrylcholinesterase
IMS	immunomagnetic separation
LC-MS/MS	liquid chromatography tandem mass spectrometry
OP	organophosphorus
ChE	cholinesterase
GB	sarin
AChE	acetylcholinesterase
TNO	The Netherlands Organisation for Applied Scientific Research
Ab	antibody
QC	quality control
F	phenylalanine
G	glycine
E	glutamic acid
S	serine
A	alanine
LRL	lowest reportable limit
QCH	quality control high
QCL	quality control low

MRM	Multiple Reaction Monitoring
LOD	limit of detection
LOQ	limit of quantitation
RSD	relative standard deviation
CLSI	Clinical and Laboratory Standards Institute
MeP	methylphosphonic acid

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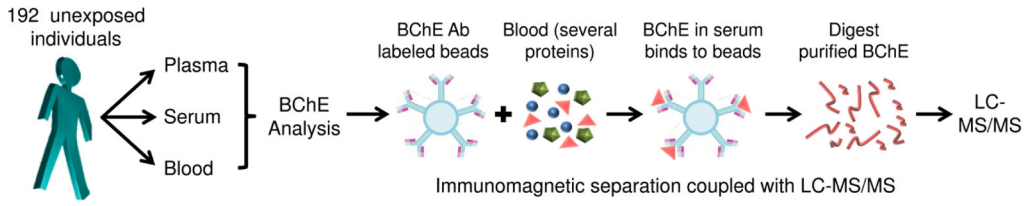

Fig. 1.
IMS Coupled with LC-MS/MS Methodology

(A)

Analyte	Peptide Sequence	Precursor Ion Q1 *(m/z)*	Product Ion Q3 *(m/z)*	DP (V)	EP (V)	CE(V)	CxP (V)
BChE_Q	$FGES_{198}AGAAS$	796.3	691.3	136	10	41	52
BChE_C	$FGES_{198}AGAAS$	796.3	620.3	136	10	37	54
BChE_ISTD	$^{13}C_9$-$FGES_{198}AGAAS$	805.4	700.3	86	10	39	54
GB-BChE_Q	$FGES_{198}$(-GB)AGAAS	916.4	778.3	106	12	45	22
GB-BChE_C	$FGES_{198}$(-GB)AGAAS	916.4	673.3	106	8	45	46
GB-BChE_ISTD	$^{13}C_9$-$FGES_{198}$(-GB)AGAAS	925.4	787.4	106	10	46	20
VX-BChE_Q	$FGES_{198}$(-VX)AGAAS	902.3	778.3	144	14	41	31
VX-BChE_C	$FGES_{198}$(-VX)AGAAS	902.3	673.3	96	4	41	31
VX-BChE_ISTD	$^{13}C_9$-$FGES_{198}$(-VX)AGAAS	911.4	787.4	120	6	40	20

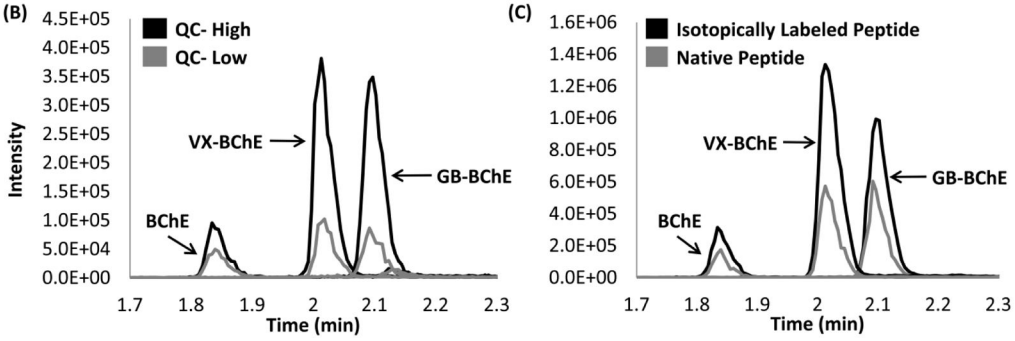

Fig. 2.

HPLC-MS/MS chromatograms and transitions. (A) Multiple Reaction Monitoring (MRM) Transitions for HPLC-MS/MS analysis of the analytes. An overlay of the reconstructed MRM chromatograms for (B) QC material and (C) native and isotopically labeled synthetic peptides. Q= Quantitation ion; C= confirmation ion; ISTD; Internal standard; DP= declustering potential; EP= entrance potential; CE= collision energy; CxP= collision cell exit potential

Table 1

Calculated Limits and Precision of Calibrators (N=20).

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Mean RSD (%)
BChE	1.42	4.73	8.13
BChE_C	1.34	4.47	5.74
GB-BChE	0.79	2.63	6.13
GB-BChE_C	0.69	2.30	6.03
VX-BChE	0.43	1.43	5.44
VX-BChE_C	0.39	1.30	5.80

BChE= quantitation ion for BChE analyte; BChE_C= confirmation ion for BChE analyte

Table 2

Interday Precision of QC Materials (N=20) Over 5 Months.

Quality Control Serum	Calculated Conc. (± SD) (ng/mL)	RSD (%)
QCH BChE	39.44 (± 3.44)	8.60
QCL BChE	21.46 (±2.14)	9.70
QCH GB	40.92 (±3.15)	7.77
QCL GB	10.43 (±1.01)	9.18
QCH VX	41.16 (±3.13)	8.71
QCL VX	8.38 (± 1.55)	18.88

QCL= Quality Control Low, QCH= Quality Control High

Table 3

Optimized IMS Protein Recovery of BChE from Serum

	Previous Method (% Total)	Current Method (% Total)		
Depletion Number	**QCH BChE**	**QCH BChE**	**QCH GB**	**QCH VX**
1	53.4	88.2	92.2	95.4
2	38.0	6.8	6.5	4.6
3	8.6	5.0	1.3	0.0

Table 4

Convenience set from 192 matched-set plasma, serum and blood samples.

Matrix	BChE Conc. (± SD) (ng/mL)	BChE Range(ng/mL)	%RSD
Plasma	34.8 (± 7.8)	16.3–58.3	22.41
Serum	34.0 (± 9.2)	13.8–57.7	27.06
Blood*	36.0 (±7.7)	16.5–57.7	21.39

*Plasma was collected from blood in-house immediately after arrival